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The influence of the rearing period on intramammary infections in Swiss dairy heifers: A cross-sectional study

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ABSTRACT

Healthy replacement heifers are one of the foundations of a healthy dairy herd. Farm management and rearing systems in Switzerland provide a wide variety of factors that could potentially be associated with intramammary infections (IMI) in early lactating dairy heifers. In this study, IMI with minor mastitis pathogens such as coagulase-negative staphylococci (CNS), contagious pathogens, and environmental major pathogens were identified. Fifty-four dairy farms were enrolled in the study. A questionnaire was used to collect herd level data on housing, management and welfare of young stock during farm visits and interviews with the farmers. Cow-level data such as breed, age at first calving, udder condition and swelling, and calving ease were also recorded. Data was also collected about young stock that spent a period of at least 3 months on an external rearing farm or on a seasonal alpine farm. At the quarter level, teat conditions such as teat lesions, teat dysfunction, presence of a papilloma and teat length were recorded. Within 24 h after parturition, samples of colostral milk from 1564 quarters (391 heifers) were collected aseptically for bacterial culture. Positive bacteriological culture results were found in 49% of quarter samples. Potential risk factors for IMI were identified at the quarter, animal and herd level using multivariable and multilevel logistic regression analysis. At the herd level tie-stalls, and at cow-level the breed category "Brown cattle" were risk factors for IMI caused by contagious major pathogens such as *Staphylococcus aureus* (*S. aureus*). At the quarter-level, teat swelling and teat lesions were highly associated with IMI caused by environmental major pathogens. At the herd level heifer rearing at external farms was associated with less IMI caused by major environmental pathogens. Keeping pregnant heifers in a separate group was negatively associated with IMI caused by CNS. The odds of IMI with coagulase-negative staphylococci increased if weaning age was less than 4 months and if concentrates were fed to calves younger than 2 weeks. This study identified herd, cow- and quarter-level risk factors that may be important for IMI prevention in the future.

1. Introduction

It is well accepted that good udder health is crucial for the economic success of a dairy farm. However, farmers often pay less attention to the rearing of young stock than to the management of adult cows, even though it has been shown that adequate management of young stock can avoid future udder health problems (Le Cozler et al., 2008). Recent studies distinguish between clinical and subclinical heifer mastitis, depending on the presence or absence of inflammatory signs in the mammary gland (Piepers et al., 2010). Heifer mastitis is a disease which may be increasing in importance in different parts of the world. In New Zealand, 21.5% of quarters of heifers had a positive bacterial culture result (Compton et al., 2007) and in a Belgium study 25% of quarters of early postpartum heifers were culture positive (Piepers et al., 2010). Although CNS is the most frequently isolated pathogen in heifers (Fox, 2009; Piepers et al., 2011) CNS is traditionally categorized as minor pathogen and only in rare cases results in clinical mastitis in heifers (Lam et al., 1997). Piepers et al. (2011) reported that CNS infection in heifers in early lactation was very common (72% of tested quarters

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Table 1A
Description of herd level risk factors potentially related to intramammary infections in Swiss dairy heifers.

Independent variable	Categories	Definition of categories
Variables at herd level (demographic data)		
Herd size	12–24 dairy cows	Tercile 1
	24–33 dairy cows	Tercile 2
	34–115 dairy cows	Tercile 3
Geographical region of the dairy farm (Cadastral zones[1])	Lowland zone	Territorial division of agricultural area with different climate, infrastructure and surface structure
	Mountain zone I	
	Mountain zone II	
	Mountain zones III and IV	
Average milk production in year 2012	5500–7000 kg	Tercile 1
	7000–7800 kg	Tercile 2
	7800–10,000 kg	Tercile 3
Yield corrected herd somatic cell count CHSCC1	<100,000 (cells/mL)	Average in the year 2012
	≥100,000 (cells/mL)	
Yield corrected herd somatic cell count CHSCC2	<200,000 (cells/mL)	Average in the year 2012
	≥200,000 (cells/mL)	
Housing system (Dairy cows)	Loose housing	
	Tie-stall barn	
Housing young stock		
Housing of calves	Crate	
	Igloo	
	Group pen	
Housing of young cattle	Tie-stall barn	
	Deep straw grouped	Deep straw bedded group pens without cubicles
	Free-stall with cubicles	Free-stall with cubicles
	Tie-stall barn	Tie-stall barn
Alpine rearing	Yes/No	Communal alpine pasturing during summer
External rearing	Yes/No	Raising in specialized farms with animals of other farms
Feeding of rearing cattle		
Period of milk feedin	<4 months	Tercile 1
	4 months	Tercile 2
	>4 months	Tercile 3
Amount of whole milk fed	L//day	Range: 5–8 l/day
Quality of whole milk fed	Milk with antibiotic residues	
	High SCC milk	
	Bulk tank milk	
Feeding of minerals to calves	Yes/No	
Calf age at the start of additional feeding	Directly after birth	Tercile 1
	After 1 week	Tercile 2
	After 2 weeks	Tercile 3
Feeding concentrates for calves	Yes/No	
Type of roughage for cattle	Only hay	
	Second cut hay	
	Corn Silage	
Feeding concentrates to heifers	Grass silage	
Feeding of minerals to heifers	Yes/No	
Grazing regimen	Yes/No	
	<6 months	Tercile 1
	6–7 months	Tercile 2
	>7 months	Tercile 3
Heifer management		
Preconditions for the first insemination	Age	
	Weight	
	Development	
	Season	
Desired calving age of heifers	24–26 months	
	27–29 months	
	≥30 months	
Adaption time in the productive herd	<2 weeks	Tercile 1
	2–3 weeks	Tercile 2
	>3 weeks	Tercile 3
Heifers housed with dry cows	Yes/No	

infected) and was associated with fewer cases of clinical mastitis (CM) throughout the following lactation compared to non-infected herd mates. In Piepers' study the occurrence of IMI caused by contagious pathogens such as *S. aureus* and *Streptococcus agalactiae (S. agalactiae)*, and environmental pathogens such as *Streptococcus uberis (S. uberis), Streptococcus dysgalactiae (S. dysgalactiae)* and *Escherichia coli* (*E. coli*) were less prevalent in early lactation heifers.

Several studies have identified potential risk factors for heifer mastitis (De Vliegher et al., 2004; Svensson et al., 2006; Piepers et al., 2011; De Vliegher et al., 2012; Krömker et al., 2012; Archer et al., 2013; Bludau et al., 2014; Abb-Schwedler et al., 2014). It is reported to be a multifactorial disease influenced by climate, season, geographical location and genetic background. In particular management factors such as social stress, type of housing sys-

Table 1B
Description of cow- and quarter-level risk factors potentially related to intramammary infections in Swiss dairy heifers.

Independent variable	Categories	Definition of categories
Variables at quarter level		
Teat lesions	Yes/no	Presence of lacerations and bruises
Teat swelling	Yes/no	Swollen teats
Papilloma	Yes/no	Presence of papilloma on the teat skin
Dysfunction or abnormality of the teat	Yes/no	Atrophy of teat canal
Teat length	Short teats	<5 cm
	Normal teats	5–7 cm
	Long teats	>7 cm
Variables at heifer level		
Breed	Holstein	Holstein cows with and without recessive red factor, Red Holstein
	Brown cattle	Swiss brown cattle, Brown Swiss, Original brown cattle
	Red pied	Simmental, Swiss Red Pied, Montbéliarde
Age at calving	Early calving age	<24 months
	Middle calving age	25–30 months
	Late calving age	>30 months
Season of calving	Winter	January–March
	Spring	April–June
	Summer	July–September
	Autumn	October–December
Progress of parturition	Normal	No assistance
	Dystocia	Assistance of >1 person
	Stillbirth	If calf was born dead or died within 48h
Assisted calving	Yes/No	
General condition of the heifer	Good	animal is attentive, standing, eats normal
	Slightly disturbed	animal is attentive, standing, does not eat
	Seriously disturbed	animal does not get up and is anorexic
Udder condition (Scores)	Soft	
	Swollen, firm	
	Red, swollen, firm, warm	
Udder edema	Yes/No	Retained fluids in the intracellular spaces of mammary tissue
Milk flow (Score) assessed by hand milking	High milk flow	Easy milker
	Normal milk flow	
	Low milk flow	Hard milker

tem, lack of environmental hygiene or inadequate nutrition have been associated with IMI in first lactation heifers (Svensson et al., 2006; Nyman et al., 2009; Santman-Berends et al., 2012). Depending on the housing and management system, heifers may suffer from social stress during multiple group changes around calving; for example being moved from the pregnant heifer group to the dry cow group, then to the transition cow group and finally to a lactating cow group. In addition to these social challenges, heifers must deal with physiological changes related to growing, calving and the first lactation. Collectively these factors can negatively influence their immune system and elevate the risk for all infections, including IMI (Mallard et al., 1998; Hultgren and Svensson, 2009).

In Switzerland, a variety of farm management systems exist, including loose housing systems and traditional tie-stall systems. There is a high degree of animal movement in Switzerland due to young stock rearing on specialized farms in which animals from different farms are comingled (Gloor et al., 2007). Calves are moved to external rearing farms at weaning age and are kept there until shortly before calving. Approximately 25% of young stock (personal communication S. Schärrer, Food Safety and Veterinary Office Switzerland, FSVO) are sent to communal alpine pastures in the summer (June to September) where animals from many different herds of origin are comingled. Mountain pastures offer some challenges to heifers such as the variable nutrient composition of the pasture, steep and heterogeneous topography and a colder, more variable climate than in the lowlands (Ruhland et al., 1999; Leiber et al., 2006). On the other hand, alpine grazing of the young stock has been reported to strengthen the health of heifers. (Kuenzi et al., 1988; Ruhland et al., 1999). Young animals are challenged by transport stress, social stress when introduced to a new group, exposure to a new microbiological flora in the new environment and exposure to the risk of transmission of mastitis pathogens through inter-suckling or flies (De Vliegher et al., 2012).

Most of the heifer mastitis studies reported in the literature focus on the period around calving (De Vliegher et al., 2004; Nyman et al., 2009; Piepers et al., 2011; Krömker et al., 2012). To our knowledge, only one Swedish study reported different factors associated with the entire rearing period (Hultgren and Svensson, 2009). The purpose of our study was to address this gap by investigating potential associations between herd, animal, and quarter level factors present during the rearing period, and the occurrence of heifer mastitis in Swiss dairy heifers.

2. Materials and methods

The study was conducted in accordance with the animal welfare legislation of Switzerland and all ethical aspects of the study were approved by the Federal Food Safety and Veterinary Office.

2.1. Herd selection and sample size calculation

All herds included in the study were affiliated with one of the main Swiss breeding organizations: Swiss Brown Cattle Breeders' Federation, Zug, Switzerland; Holstein Breeders' Federation, Posieux, Switzerland; and the Swissherdbook, Zollikofen, Switzerland. Herds were included if they had at least 20 lactating cows of one of the breeds: Holstein Friesian, Red Holstein, Brown Swiss, Original Brown, Braunvieh (BS × Original Brown), Swiss Red Pied, Simmental or Montbéliarde, and were located at the north side of the Alps. Participation in the study was voluntary. Owners of 856 farms were invited to participate in the study, of these, 224 farm owners agreed to participate, and 72 of these farms were randomly selected for inclusion in the study, using the RAND function in Excel. Piepers et al. (2011) reported 20% loss of samples due to contamination. In anticipation of this potential loss we adjusted our sample size accordingly. Our farm sample size was estimated to be 60 farms and was adjusted to 72 farms to account for these poten-

Table 2
Definition of infection status of quarters for any of the pathogen groups considered.

Culture result ofDuplicate sample 1	Culture result ofDuplicate sample 2	Infection status of quarter
positive	positive	infected
positive	negative	infected
negative	positive	infected
negative	negative	healthy
positive	contaminated[a]	infected
contaminated	positive	infected
negative	contaminated	healthy
contaminated	negative	healthy
contaminated	contaminated	exclusion

[a] More than 2 different pathogens present.

tial sample losses ($60 \times 1.20 = 72$). We estimated an average of 6 heifers per farm, providing a minimum sample size of around 1440 ($60 \times 6 \times 4 = 1440$) quarters. Logistic regression of a binary response variable (Y) on a binary independent variable (X) with a sample size of 1564, (final sample of quarters, of which 50% are in the group X = 0 and 50% are in the group X = 1) was calculated to have 78% power, at a 0.05 significance level (Hsieh et al., 1998; PASS Software, 2014).

2.2. Data collection

The 72 selected dairy farms, together with their cooperating 54 heifer rearing operations, and 33 alpine farms where the cattle spend the summer period, were visited one time between May 2012 and August 2013. Demographic farm data and data about management practices were collected using a questionnaire during a farmer interview conducted by a research team member while visiting the farm. During the farm visit, animals, barns and pastures were inspected and evaluated for hygiene and animal welfare. Variables collected at the herd, heifer- and quarter-level can be found in Tables 1A and 1B.

During farm visits dairy farmers were trained to aseptically collect milk samples according to the guidelines of the National Mastitis Council (National Mastitis Council, 2004). There is no consensus concerning the case definition of IMI. In a New Zealand study, IMI status was assessed with duplicate samples collected at one time point (Compton et al., 2007) whereas in two Belgium studies several consecutive samples were collected to define IMI status with different types of bacteria (Piepers et al., 2010; Piepers et al., 2011). We chose to use duplicate samples from each quarter at the time of calving, and farmers were instructed to aseptically collect duplicate individual quarter milk samples from each heifer immediately after parturition for the duration of the study. All heifers that calved were scored for cow-level risk factors immediately after parturition by farmers All milk samples were frozen immediately after collection. Completed score sheets and frozen milk samples were sent by priority mail to the laboratory the day after collection (ILS, Institute for Food Safety and Hygiene, Zurich, Switzerland). Transport duration was a maximum of 24 h and samples were shipped in an insulated styropor box to prevent heat damage. The sampling period was from May 2012 until October 2013.

2.2.1. Herd-level

Herd-level data including demographic information, housing, management and feeding of young stock were collected during an interview with the farmer (Table 1B). Variables originally recorded as continuous data were transformed to categorical variables using their terciles to define three categories. Herd size, average milk production per cow and year and average yield corrected herd somatic cell count (CHSCC) for the 2012 calendar year were calculated from dairy herd improvement (DHI) data obtained from the Swiss breeding organizations, as described by Lievaart et al. (2007) and Ivemeyer et al. (2009).

2.2.1.1. Assessment of the welfare status. Most published protocols for assessing cattle welfare focus on the welfare of producing dairy cattle or fattening cattle. For this reason a specific protocol for evaluation of welfare in rearing dairy cattle was developed for this study (see Supplementary online material).

Inspired by the work of Bartussek (1996a,b), Sundrum (2007), Schaeffer et al. (2007), Von Borell et al. (2007), von Keyserlingk et al. (2009) and the Welfare Quality Assessment protocol for cattle (2009), five key areas relating to the natural behaviour of animals were considered: (1) assessment of locomotion area (supplementary material Table S1), (2) assessment of resting area (supplementary online material table S2), (3) assessment of feeding area and feeding management (supplementary online material, table S3), (4) assessment of general climatic aspects (supplementary online material, table S4), (5) assessment of general animal health and hygiene (supplementary online material, table S5). A total of 20 score points were allocated to each key area providing a maximum of 100 score points.

Welfare was assessed separately in each of the following 5 calf and heifer groups: (1) calves up to 3 weeks of age, (2) calves 4–8 weeks of age, (3) calves 9–15 weeks of age, (4) young prepuberal heifers and (5) postpuberal and pregnant heifers.

2.2.2. Cow-level

A score sheet for recording information about calving ease, udder health, general health and milking characteristics of the heifer was completed by the farmer at the time that milk samples were collected. Cow-level variables are reported in Table 1A. Variables originally recorded as continuous were transformed into categorical variables with three categories using their terciles.

2.2.3. Quarter-level

Quarter-level data included teat lesions, teat swelling, presence of papilloma, and teat length (categorized as: long = >7 cm, medium = 5–7 cm, short = <5 cm) were recorded immediately after parturition by measuring the length from the teat tip to the base with a measuring tape (Table 1A). Teat dysfunctions, such as teat lacerations or additional teat canals with or without gland tissue were also recorded.

2.2.3.1. Bacteriological analysis and definition of IMI-Status. Bacteriological analysis was performed according to NMC standards (National Mastitis Council, 2004). Coagulase-negative staphylococci were not further specified. A sample with more than 2 different bacterial species was classified as contaminated and excluded from further analysis. If one of the duplicate samples was contaminated, the findings from the uncontaminated duplicates were used to identify an infection.

Quarters were divided into four groups: non-infected, infected with CNS, infected with contagious major pathogens or infected with environmental major pathogens as previously reported

Table 3
Prevalence (%) of mastitis-relevant pathogens in 391 heifers and their 1564 quarters presented at the cow- and quarter-level.

	Non- infected	Contagious*S. aureus*	Environmental[a]	CNS[b]	Others[c]	In total
Number of quarters	767	75	95	664	10	1564
Prevalence (%)	49.0	4.8	6.1	42.5	0.6	[d]
Number of cows	96	41	75	270	7	391
Prevalence (%)	24.6	10.5	19.2	69.1	1.8	[d]

[a] Environmental: Coliforms, *S. dysgalactiae, S. uberis*.
[b] CNS: Coagulase- negative staphylococci.
[c] The category includes *Trueperella pyogenens* (*T. pyogenes*), aerobic spore formers, *Corynebacterium bovis* (*C. bovis*) and other Gram-positive rods.
[d] The sum of all percentages is bigger than 100% because cows can have two pathogens at one quarter.

Table 4
Number of farms and prevalence (%) of mastitis-relevant pathogens in heifers, at the herd level in 54 Swiss dairy farms.

Category	*Number* of farms (%)	Classification	% infected heifers/farm
S.aureus	39 (72)	all heifers negative	0
	15 (28)	>1 heifer positive	7–78
Environmental[a]	20 (37)	Environmental pathogen free	0
	17 (32)	Intermediate germ exposure (<25%)	7–22
	17 (32)	High pathogen exposure (>25%)	25–80
CNS[b]	9 (17)	Low CNS exposure (0–50%)	8–44
	15 (28)	Intermediate CNS exposure(50–75%)	50–71.
	30 (56)	High CNS exposure (>75%)	75–100

[a] Environmental: Coliforme bacteria, *S. uberis*, *S. dysgalactiae*.
[b] CNS: Coagulase- negative staphylococci.

Table 5
Medians and 95% CI of total welfare scores for young stock at the herd level in 54 Swiss dairy farms.

Category	Median	95% CI	Maximum score	Farms ≥80 (%)[a]	Farms <50 (%)[b]
Calf group1	67.4	45.5–85	100	9 (16.7)	6 (11.1)
Calf group 2	78	58–94	100	25 (46.3)	0 (0)
Calf group 3[c]	74	58–89	100	15 (27.8)	1 (1.9)
Heifer group 1	78	61.5–88	100	20 (37.0)	0 (0)
Heifer group 2	78	61.5–89	100	24 (44.4)	0 (0)
Total Score			500		

[a] More or equal 80% of maximum points was interpreted as good welfare.
[b] Less than 50% of maximum points was interpreted as decreased welfare.
[c] Four farms have no calf group 3. Since calf group 3 was integrated in calf group 2 on these 4 farms, the total score of was calculated using a double score of calf group 2.

(Piepers et al., 2011). Definition of the infection status of quarters using duplicate samples is explained in Table 2.

2.3. Statistical analyses

The udder quarter was the unit of this analysis. To take into account clustering of quarters within heifers and heifers within herds, heifer and herd were included as random effects in the multilevel models. Three-level logistic regression mixed models with random intercepts were fit using Stata 13. The log likelihood for this type of model has no closed form, so it was approximated by adaptive Gaussian quadrature (StataCorp., 2013a,b). Prior to multivariable analysis, univariable three-level models were used to test the associations between the binary outcome variables (a) IMI with contagious major pathogens (1 = infected; 0 = non-infected), (b) IMI with CNS (1 = infected; 0 = non-infected), (c) IMI with environmental major pathogens (1 = infected; 0 = non-infected) and potential risk factors. Variables with $P < 0.20$ were kept for further analysis. Collinearity between potential risk factor variables was evaluated using Pearson's and Spearman's rank correlation. If two risk factors had a correlation coefficient of >0.60, the one with the lower P-value in the univariable analysis and considered biologically more plausible was included in the multivariable model. For each outcome (a–c), a separate multivariable model was built, following the recommendations of the online course of the Centre for Multilevel Modelling, Bristol, UK (http://www.bristol.ac.uk/cmm/learning/online-course/index.html). We first computed the null models, and compared them with models that included every single explanatory variable, one at a time. Likelihood ratio (LR) tests, which do not rely on the assumption of an asymptotic normal sampling distribution, were used to prove that the additional predictors significantly improved the fit of the models. We then proceeded to build more complex models adding one variable at a time and running successive Likelihood Ratio (LR) tests. We used a significance level of $P < 0.05$ to decide which variables remained in the final models. This model selection procedure accounts, at the same time, as a control for confounding, because any variable that renders a significant LR test, and thus influences the model, is retained. Every three-level model was compared with its single-level model counterpart using LR-tests and found to significantly better fit the data.

The Variance Partition Coefficients (VPC) of IMI with contagious and environmental major pathogens and CNS at the herd, heifer and quarter level for both null and final models were estimated. The variance at the quarter level was fixed (constant) to $\pi^2/3 = 3.29$ (with $\pi = 3.1416$) for the logistic model (Dohoo et al., 2001; Snijders and Bosker, 2012). Intraclass correlation coefficients (ICCs) that measure the similarity of the observed responses within a given level or cluster were also calculated.

3. Results

3.1. Description of the sample

Colostrum samples were collected from 528 heifers on the original 72 farms. Eighteen farms left the study before it was

Table 6A
Number and proportion of heifers and quarters for potential heifer- and quarter-level risk factors included in the analyses per pathogen. Only those with a P-value ≤ 0.2 were included in the multivariable models. For variables with more than two categories, the first category is the reference.

Independent variable	N (%) of quarters	Selected for multivariable analysis		
	1564 quarters	*S. aureus*[a] (*P-value*)	Environ.[b] (*P-value*)	CNS 3[c] (*P-value*)
Quarter-level				
Teat lesions (yes)	7 (0.45)	No (0.406)	Yes (0.062)	Yes (0.005)
Teat swelling (yes)	164 (10.48)	No (0.734)	Yes (0.011)	No (0.574)
Papilloma (yes)	101 (6.45)	No (0.327)	Yes (0.123)	No (0.660)
Dysfunction or abnormality of the teat (yes)	15 (0.95)	No (0.747)	Yes (0.011)	No (0.467)
Teat length		No (0.071)	No (0.169)	No (0.048)
short	446 (28.51)			
middle	1090 (69.69)			
long	28 (1.79)			
Heifer-level				
Breed		Yes (<0.001)	Yes (0.083)	Yes (0.165)
Holstein	384 (24.55)			
Brown cattle	676 (43.22)			
Red pied	496 (31.71)			
Mixed breed	8 (0.51)			
Age at 1st calving		No (0.981)	No (0.299)	Yes (<0.001)
Early calving age	96 (6.13)			
Typical calving age	824 (52.67)			
Late calving age	644 (41.17)			
Calving season		Yes (<0.001)	Yes (0.002)	Yes (<0.001)
Winter	304 (19.43)			
Spring	156 (9.97)			
Summer	480 (30.69)			
Autumn	624 (39.89)			
Calving ease		Yes (<0.001)	No (0.287)	Yes (0.029)
Normal	1408 (90.03)			
Difficult (dystocia or stillbirth)	156 (9.97)			
Assisted calving (yes)	616 (39.38)	Yes (0.001)	Yes (0.024)	No (0.317)
General condition of the heifer (good)	1532 (97.95)	Yes (0.075)	No (0.967)	Yes (0.113)
Udder swelling (yes)	684 (43.73)	No (0.601)	Yes (0.159)	Yes (0.194)
Udder edema (yes)	526 (33.63)	No (0.575)	No (0.648)	No (0.612)
Milk flow "easy milker" (yes)	132 (8.43)	Yes (0.079)	Yes (<0.001)	No (0.448)

[a] Dependent variable "Intramammary infection with *Staphylococcus aureus* pathogen".
[b] Dependent variable "Intramammary infection with environmental major pathogen".
[c] Dependent variable "Intramammary infection with coagulase-negative staphylococci".

completed. Twelve of these were excluded because of too few submitted samples, one farm because of contamination of samples, one farm because of contamination of samples and too few submitted samples, and 4 farms had less than 3 heifers that calved during the sampling period. The mean herd size was 31 cows (SD = 16). The mean milk yield per herd was 7438 kg/cow/year (SD = 940 kg/cow/year). Twenty-five farms (46%) had loose housing systems, 28 farms (52%) had tie-stalls and one had a mixed system. Twenty-six farms (48%) were located in the lowlands (cadastral zone: lowland), and 28 farms (52%) were located in mountainous areas (cadastral zones: mountain zone1-4). The mean CHSCC in the year 2012 was 134,000 cells/mL (SD = 72,000 cells/mL). Young stock from 22 dairy farms (41%) were reared at specialized rearing farms together with animals from other farms. Thirty-two dairy farms (59%) housed their young stock at the farm of origin. The young stock of 38 farms (70%) were sent to alpine pastures during the summer.

3.2. Results of the bacteriological examination

The complete spectrum of detected mastitis pathogens is presented in Tables 3 and 4. A total of 1564 quarters were bacteriologically examined and in 51.0% (n = 797) at least one pathogen was detected (Table 3); 42.5% were infected with CNS, 6.1% with one or two environmental major pathogens (3.2% *S. uberis*, 1.9% coliforms and 1.2% *S. dysgalactiae*) and 4.8% with a contagious major pathogen. Pathogens categorized as contagious major pathogens (in this study only *S. aureus*) were found in 10.5% of the heifers and in 15 of 54 (27.8%) dairy farms. Environmental major pathogens were found in 19.2% of the heifers and 34 dairy farms (63%). Coagulase-negative staphylococci were diagnosed in 69.1% of the heifers; there was no farm free of CNS.

3.3. Results of the farmís welfare status

Farms frequently had deficiencies in the key areas of locomotion area and feeding infrastructure and management (Table 5). There were correlations between the welfare scores of different groups, and for this reason only the total score was tested in the final model.

3.4. Risk factor analysis

3.4.1. Univariable analysis

The results of the univariable analysis are presented in Tables 6A–D, which includes the variables that were considered in the final models. The individual SCC of the heifers at first test day was associated with the prevalence of environmental major pathogens only ($P = 0.003$), and not with the prevalence of contagious major pathogens ($P = 0.65$) or CNS ($P = 0.29$). The association was, however, weak and not significant in the final multivariable model.

3.4.2. Multivariable multilevel logistic regression models and analysis of variance

3.4.2.1. Model Staphylococcus aureus. Heifers of the breed category "Brown cattle" were more likely to have IMIs with *S. aureus* (odds ratio; OR 11.2) than the other breeds. Heifers housed in tie-stalls (OR 26.9) were more likely to have IMIs with *S. aureus* than heifers in loose housing systems. The results of the multivariable

Table 6B
Number and proportion of herds for all potential herd-level risk factors included in the analyses per pathogen. Only those with a P-value ≤ 0.2 and were included in the multivariable models. For variables with more than two categories, the first category is the reference. Part I.

Independent variable	N (%) of quarters	Selected for multivariable analysis		
	1564 quarters	*S. aureus*[a] (*P-value*)	Environ.[b] (*P-value*)	CNS 3[c] (*P-value*)
Herd-level				
Herd size (terciles)		No (0.950)	No (0.717)	No (0.300)
Tercile 1 (12–24 dairy cows)	528 (33.76)			
Tercile 2 (25–33 dairy cows)	500 (31.97)			
Tercile 3 (34–115 dairy cows)	536 (34.27)			
Geographical region of the dairy farm (Cadastral zones)		Yes (<0.001)	Yes (0.003)	Yes (0.075)
Lowland zone	668 (42.71)			
Mountain zone I	360 (23.02)			
Mountain zone II	376 (24.04)			
Mounain zone III and IV	160 (10.23)			
Average milk production in 2012		Yes (<0.001)	Yes (0.061)	No (0.395)
Tercile 1 (low)	512 (32.74)			
Tercile 2 (intermedium)	600 (38.36)			
Tercile 3 (high)	452 (28.90)			
Yield corrected herd somatic cell count (CHSCC)		Yes (0.002)	Yes (0.009)	Yes (<0.001)
<100,000 (cells/mL)	432 (27.62)			
≥100,000 (cells/mL)	1132 (72.38)			
Housing system (Dairy cows)		Yes (<0.001)	No (0.451)	Yes (0.076)
Loose housing	724 (46.29)			
Stanchion barn	820 (52.43)			
Mixed system	20 (1.28)			
Housing calf group 1		Yes (<0.001)	Yes (0.091)	Yes (0.011)
Crate	508 (32.48)			
Igloo	332 (21.23)			
Calf pen	644 (41.18)			
Mixed system	40 (2.56)			
Housing calf group 2		Yes (<0.001)	Yes (0.151)	Yes (<0.001)
Crate	28 (1.79)			
Igloo	208 (13.30)			
Calf pen	956 (61.13)			
Loosing housing	244 (15.60)			
Mixed system	128 (8.18)			
Housing calf group 3		Yes (0.126)	No (0.978)	Yes 0.025)
Calf pen	76 (4.86)			
Tie-stall	448 (28.64)			
Loose housing	860 (54.99)			
Mixed system	180 (11.51)			

[d] Farmers who fed high SCC milk often fed milk containing antimicrobial residues, too.
[a] Dependent variable "Intramammary infection with contagious major pathogen".
[b] Dependent variable "Intramammary infection with environmental major pathogen".
[c] Dependent variable "Intramammary infection with coagulase-negative staphylococci".

analysis with the corresponding 95% confidence intervals and *P*-values are presented in Table 7.

3.4.2.2. Model environmental major pathogens. Quarters with swollen teats (OR 2.67) or teat lesions (OR 37.7) were more likely to be infected with environmental major pathogens than heifers with normal teats. Teat lesions were recorded in only 7 quarters (0.4%). Heifers raised on specialized rearing farms (OR 0.29) were less likely to be infected with environmental major pathogens than heifers raised at their home farms.

3.4.2.3. Model CNS. The separation of pregnant heifers from younger replacement animals had the strongest effect on the presence of CNS, showing a protective effect against CNS IMI.

Three additional factors were associated with the presence of CNS. During model selection the Log-likelihood ratio tests demonstrated that the following 3 final models with variables were equally valid (Table 7): (1) separation of pregnant heifers and feeding concentrates to calves ($P = 0.006$), (2) separation of pregnant heifers and the weaning age ($P = 0.002$) and (3) separation of pregnant heifers and welfare of calves ($P = 0.003$), although this last model had a very small effect with an OR very close to one. A special group for heifers decreased the odds of CNS infection in each of the 3 models (OR 0.2–0.32). Feeding concentrates to replacement calves younger than 2 weeks and weaning calves before 4 months of age increased the odds of CNS infection.

3.4.2.4. Variance components. For contagious pathogens i.e. *S. aureus*, the random effect variance was more evenly distributed in the three levels than for CNS and environmental pathogens (Table 8), for which most of the unexplained variance remained at the levels of quarter and cow. In the final model, the random variance at herd level still amounts to 24% for contagious pathogens. Two farms stood out with a very high proportion of infected quarters (15 and 19 respectively of the total of 75 infected quarters in all farms).

The ICC at the farm level was higher for contagious (24%) than for CNS (5–6%) or environmental (7%) major pathogens (Table 9). A similar pattern was found at the cow level but with much higher ICC values, 70%, 56% and 51%, for contagious, environmental, and CNS, respectively.

4. Discussion

4.1. *Prevalence of* Staphylococcus aureus *and its risk factors*

In this study the "housing system" was strongly associated with the prevalence of *S. aureus* IMI at calving. The prevalence of IMI at calving was higher in heifers housed in tie-stall barns, which, in

Table 6C
Number and proportion of herds for all potential herd-level risk factors included in the analyses per pathogen. Only those with a P-value ≤ 0.2 and were included in the multivariable models. For variables with more than two categories, the first category is the reference. Part II.

Independent variable	N (%) of quarters	Selected for multivariable analysis		
	1564 quarters	*S. aureus*[a] (P-value)	Environ.[b] (P-value)	CNS 3[c] (P-value)
Herd-level				
Housing heifer group 1		Yes (<0.001)	No (0.362)	Yes (0.034)
Deep house without cubicles	244 (15.60)			
Cubicle house for untethered cattle	664 (42.25)			
Stanchion barn	628 (40.15)			
Mixed system	28 (1.79)			
Housing heifer group 2		Yes (<0.001)	No (0.661)	Yes (0.002)
Deep-straw without cubicles	124 (7.93)			
Cubicle house for untethered cattle	780 (49.87)			
Tie-stall barn	632 (40.41)			
Mixed system	28 (1.79)			
Alpine pasturing (yes)	1120 (71.61)	No (0.017)	Yes (0.015)	Yes (0.2)
External rearing (yes)	524 (33.50)	Yes (<0.001)	Yes (0.002	Yes (0.1)
Period of milk feeding until weaning		Yes (<0.001)	No (0.733)	Yes (<0.001)
<4 months	356 (22.76)			
4 months	536 (34.27)			
>4 months	672 (42.97)			
Amount of milk feed	(continuous)	No (0.527)	No (0.408)	No (0.676)
Quality of milk feedd				
Milk containing antimicrobial Residues (yes)	616 (39.39)	Yes (0.190)	No (0.440)	No (0.227)
High SCC milk (yes)	1156 (73.91)	Yes (0.701)	No (0.850)	Yes (0.039)
Only saleable bulk milk (yes)	348 (22.25)	No (0.312)	No (0.741)	No (0.024)
Feeding of minerals to calves (yes)	512 (32.74)	Yes (0.001)	Yes (0.161)	No (0.294)
Calf age at start of additional feeding		Yes (0.042)	Yes (0.079)	Yes (<0.001)
Tercile 1 (Directly after birth)	928 (59.34)			
Tercile 2 (After 1 week)	380 (24.30)			
Tercile 3 (After 2 weeks)	220 (14.07)			
Various	36 (2.30)			
Feeding concentrates for calves (yes)	1316 (84.14)	Yes (0.003)	Yes (0.003)	Yes (<0.001)

Switzerland, are often older than loose housing barns. Although *S. aureus* is classified as an udder-associated pathogen and the primary reservoir is the bovine udder, it has been reported that some mastitis causing strains can be found on body sites close to the udder and in the immediate environment of the cow (Anderson et al., 2012). Recently, strains of *S. aureus* causing persistent IMI were reported to have the ability to form biofilms (Veh et al., 2015). Therefore, these pathogens may persist in the environment forming reservoirs more often in old barns, which are usually more difficult to clean. The finding that heifers of the breed category "Brown cattle" were more often infected with contagious major pathogens confirmed a previous Swiss study (Ivemeyer et al., 2009), which found an association between poor udder health and the breed category "Brown cattle." "Brown cattle" farms are traditionally located in Eastern Switzerland, where communal alpine farms tend to be bigger than the ones in central and western Switzerland and are supplied by a high number of different farms of origin. Communal alpine pasturing has previously been reported to be a risk for new infections with *S. aureus* (Voelk et al., 2014).

The rare occurrence of *S. aureus* compared to environmental mastitis pathogens in the present study confirms the results of Kretzschmar et al. (2013) who investigated mastitis management in Swiss dairy farms. In our study, only 15 farms with *S. aureus* infected heifers were identified, and therefore the results of this study relating to *S. aureus* infection should be interpreted with care. It is possible that some IMI with *S. aureus* may have been missed in our study. The sensitivity of bacterial culture from a single sample using an inoculum of 0.1 ml is reported to be low (74.5%) (Sears et al., 1990). The sensitivity for detection of *S. aureus* in our study may have been higher than reported by Sears et al. (1990) because duplicate samples were collected. However, the duplicate samples were both collected at one time point and in many cases one sample was contaminated and excluded from our analysis and only a standard inoculum of 0.01 ml was used for culture. This has been reported to increase the risk of obtaining false negative results (Sears et al., 1990).

Thirty-five of the 75 quarters infected with contagious mastitis pathogens were found at two farms with a known high rate of infection with *S. aureus*. Both farms were located in Eastern Switzerland and had "Brown cattle" breed cattle which were housed in tie-stalls. One of these farms practiced communal alpine farming during the summer with all lactating cows and heifers being moved to a communal pasture. With the results of the present study it is not possible to determine which is the more important risk factor: (1) cattle of the breed "Brown cattle" being more susceptible to contagious mastitis, or (2) the management practices conducted in areas where "Brown cattle" are raised. The risk factors identified for IMI with *S. aureus* – breed "Brown cattle" and tie-stall housing – are not popular in the EU except for Northern Italy and Austria and they can be considered to be specific for pre-alpine and alpine regions. Today more than 70% of the dairy herds in EU are Holstein-Friesian (EFSA, 2009).

4.2. Prevalence of environmental major pathogens and their risk factors

For IMI with environmental pathogens, the condition of the teat (i.e. injured skin of the quarter, teat edema) has been reported to be associated with IMI. De Vliegher et al. (2004) reported that the load of bacteria at the teat end was a crucially important risk factor for IMI with environmental pathogens, and, poor heifer hygiene has been reported to be associated with CNS IMI (Piepers et al., 2011). Waage et al. (2001), reported that teat and udder edema were associated with clinical mastitis. They suggested that this may be due to impairment of blood circulation in the affected area, which in turn impairs the transport of immune cells into the affected area. Mechanical forces during milking may have a much greater effect on edematous teats than on non-edematous ones. The same study

Table 6D
Number and proportion of herds for all potential herd-level risk factors included in the different analyses. Only those with a P-value ≤ 0.2 and were included in the multivariable models. For variables with more than two categories, the first category is the reference. Part III.

Independent variable	N (%) of quarters	Selected for multivariable analysis		
	1564 in total	*S. aureus*[a] (*P-value*)	Environ.[b](*P-value*)	CNS 3[c](*P-value*)
Herd-level				
Type of roughage for heifer group1				
Silage feeding (yes)	1056 (67.52)	Yes (<0.001)	No (0.354)	Yes (0.135)
Second cut feeding (yes)	324 (20.72)	Yes (<0.001)	Yes (0.034)	No (0.339)
Full pasture grass (summer) (yes)	1292 (82.61)	Yes (<0.001)	No (0.884)	Yes (0.121)
Type of roughage for heifer group2				
Silage feeding (yes)	1092 (69.82)	Yes (<0.001)	No (0.760)	No (0.349)
Second cut feeding (yes)	196 (12.53)	Yes (<0.001)	Yes (0.040)	Yes (0.113)
Full pasture grass (summer) (yes)	1356 (86.7)	Yes (<0.001)	No (0.471)	Yes (0.04)
Feeding concentrates to cattle group1 (yes)	768 (49.10)	Yes (<0.001)	No (0.439)	Yes (<0.001)
Feeding concentrates to cattle group2 (yes)	556 (35.55)	Yes (<0.001)	No (0.694)	Yes (0.041)
Feeding of minerals to cattle (yes)	1244 (79.54)	Yes (<0.001)	No (0.692)	Yes (0.005)
Grazing regimen for cattle (per year)		Yes (<0.001)	No (0.252)	No (0.964)
Tercile 1 (<6 months)	216 (13.81)			
Tercile 2 (6–7 months)	708 (45.27)			
Tercile 3 (>7 months)	640 (40.92)			
Preconditions for the first insemination		Yes (0.008)	Yes (0.060)	Yes (0.006)
Age	248 (15.86)			
Weight/size	272 (17.39)			
Development	932 (59.59)			
Season	416 (26.60)			
Desired calving age of heifers		No (0.704)	No (0.708)	No (0.569)
Tercile 1 (24–26 months)	728 (46.55)			
Tercile 2 (27–29 months)	612 (39.13)			
Tercile 3 (≥30 months)	224 (14.32)			
Adaption time in the productive herd		Yes (<0.001)	Yes (0.130)	No (0.230)
Tercile 1 (<2 weeks)	404 (25.83)			
Tercile 2 (2–3 weeks)	404 (25.83)			
Tercile 3 (>3 weeks)	624 (39.90)			
Various	132 (8.44)			
Heifers housed with dry cows (yes)	916 (58.57)	Yes (<0.001)	No (0.517)	Yes (0.034)
Welfare Scoring – Calf group 1	(continuous)	Yes (<0.001)	Yes (0.08)	Yes (<0.001)
Welfare Scoring – Calf group 2	(continuous)	No (0.660)	No (0.574)	Yes (<0.001)
Welfare Scoring – Calf group 3	(continuous)	Yes (0.069)	Yes (0.016)	Yes (0.030)
Welfare Scoring – Heifer group 1	(continuous)	Yes (<0.001)	Yes (0.042)	Yes (0.111)
Welfare Scoring – Heifer group 2	(continuous)	Yes (<0.001)	Yes (0.024)	Yes (0.005)
Welfare Scoring – Sum of all groups	(continuous)	Yes (<0.001)	No (0.344)	Yes (<0.001)

[d]Farmers who fed high SCC milk often fed milk containing antimicrobial residues, too.
[a]Dependent variable "Intramammary infection with contagious major pathogen".
[b] Dependent variable "Intramammary infection with environmental major pathogen".
[c] Dependent variable "Intramammary infection with coagulase-negative staphylococci".

Table 7
Final multivariable models for the outcome variables intramammary infection at calving with *S. aureus*, environmental major pathogens and CNS (1564 quarters of 391 heifers at 54 farms).

Dependent variable	Independent variable	OR[a]	95% CI[b]	*P*-Value
IMI[c] with *S. aureus*	Breed "Brown cattle" (yes)	11	2.12–57.9	0.004
	Tie-stall barn (yes)	26.9	4.2–173.7	0.001
IMI with	Teat swelling (yes)	2.7	1.0–7.0	0.046
environmental	Teat lesion (yes)	37.7	2.1–690.9	0.014
major pathogens	External heifer rearing (yes)	0.3	0.1–0.7	0.005
IMI with CNS[d]	3 equally valid models:			
	Separation of pregnant heifers (yes)	0.2	0.1–0.5	0.001
	Feeding concentrates to calves<2 weeks (yes)	2.9	1.3–6.6	0.012
	Separation of pregnant heifers (yes)	0.3	0.1–0.8	0.015
	Weaning age <4months (yes)	2.2	1.0–4.5	0.041
	Separation of pregnant heifers (yes)	0.2	0.1–0.5	0.000
	Welfare score calf group 2 (1–100%)	1.04	1.0–1.1	0.003

[a]Odds ratio.
[b] 95% confidence interval.
[c] Intramammary infection.
[d] Coagulase- negative staphylococci.

reported that udder and teat edema may cause milk leakage which may be associated with clinical mastitis.

In our study heifer rearing on a specialized rearing farm was protective against environmental major pathogens. Reasons for this may be: (1) most heifer rearing farms do not keep lactating cows, so there is no exposure to infected adults, or (2) these specialized farmers pay more attention to young stock than dairy farmers who rear heifers on their own farm, because rearing for other farmers is their main income.

Table 8
Variance components at the herd, heifer and quarter levels of the null and final models for IMI with *S. aureus*, environmental major pathogens and CNS[a] (1564 quarters of 391 heifers in 54 dairy farms).

Data hierarchy	Null Model						Final Model					
	IMI with major mastitis pathogens				IMI with CNS[a]		IMI with major mastitis pathogens				IMI with CNS[b]	
	S. aureus		Environmental			*S. aureus*		Environmental				
	Var.est.	%	Var.est.	%	Var.est.[c]	%	Var.est.	%	Var.est.	%	Var.est.	%
Herd	6.05	42.8	0.48	7.1	0.74	9.6	2.61	23.9	0.27	4	0.38	5.2
Heifer	4.78	33.9	3	44.3	3.66	47.6	5	45.9	3.03	46	3.70	50.2
Quarter	3.29	23.3	3.29	48.6	3.29	42.8	3.29	30.2	3.29	50	3.29	44.6
Total variance	14.12	100	6.77	100	7.96	100	14.12	100	6.59	100	7.37	100

Note: 3.29 is per definition the variance at the lowest level for multilevel logistic regression.
[a] Coagulase-negative staphylococci.
[b] The final model with special group for heifers and feeding concentrates for calves was used for the calculation CNS IMI.
[c] Variance estimate.

Table 9
Intracorrelation coefficients for intramammary infections (IMI) with *S. aureus*, IMI with environmental major pathogens and CNS[a] (1564 quarters of 391 heifers in 54 dairy farms).

	Final model					
	IMI with major pathogens				IMI with CNS[a]	
	S. aureus		Environmental			
	Var.est.[b]	%	Var.est.	%	Var.est.[c]	%
Herd	0.24	23.9	0.04	4	0.05	5.2
Heifer	0.7	69.8	0.5	50	0.55	55.3

[a] Coagulase-negative staphylococci.
[b] Variance estimate.
[c] The final model with special group for heifers and feeding concentrates for calves was used for the calculation CNS IMI.

4.3. Prevalence of coagulase-negative staphylococci and their risk factors

The importance of CNS as a cause of bovine mastitis is still uncertain (De Vliegher et al., 2009). More than 45 different species and subspecies of the CNS group exist and 12 of them are regularly found in milk of dairy cows (Piessens et al., 2011). The pathogenicity of this group has yet to be established. It is generally accepted that major pathogens induce clinical heifer mastitis. However, IMI with CNS does not negatively influence subsequent productivity (De Vliegher et al., 2012). Our study is in agreement with other studies that reported CNS as the most prevalent mastitis pathogen in heifers (Fox, 2009; Piepers et al., 2011). In our study the presence of CNS was associated with 4 management factors: no separation of pregnant heifers, early provision of concentrates to calves, low weaning age and better welfare of calves. Separation of pregnant heifers from younger animals and adults may indicate a higher degree of professionalism in the farmer. These farmers may provide better management and feeding of young stock in order to reach set rearing targets. Weaning at an older age and feeding of concentrates later in heifer rearing are management practices that have been reported to be associated with more extensive, pasture-based rearing systems and have been reported to be protective effect against CNS IMI. The very early provision of concentrates to calves is often linked to reduced milk feeding ($\leq$10% of body weight) as reviewed by Drackley (2008), and therefore to inadequate nutrition, which might lead to insufficient development of heifers, and to an impaired immune system which may increase udder susceptibility to infection.

In contrast to other studies (Bielfeldt et al., 2006; von Keyserlingk et al., 2009) an unexpected finding in our study was, that higher welfare status of calves was associated with the presence of CNS IMI. Most of the farms in our study had welfare scores higher than 50% and the presence of CNS was not directly associated with disease.

4.4. Partition of variance components

The impact of the herd-level was more important for *S. aureus* than for the other pathogens, suggesting that there may be other herd-level risk factors not yet explored for this pathogen. The highest variation in IMI with environmental major pathogens remained at the quarter-level, and at the heifer level for CNS IMI.

The herd itself had a higher impact on the risk of IMI with contagious pathogens as previously reported for *S. aureus* (Voelk et al., 2014) and this may be explained by the probability of transmission of *S. aureus* being much higher in herds with a greater number of cows shedding the pathogen.

4.5. Strengths and limitation of this study

The case definition for IMI varies across studies and this makes comparison of study results difficult. In order to better compare study results an international consensus for the definition of IMI with different pathogens in cows and heifers is needed, based on the work of Dohoo et al. (2011) and Andersen et al. (2010). A potential sampling bias in our study could have been reduced by limiting the proximity of sampled farms to our institute and collecting data exclusively by trained veterinarians. We opted however for increased representativeness of our sample by random sampling from the volunteer farms regardless of their location. This increased representativeness allowed us to include and detect strong previously unreported risk factors, such us breed and type of housing.

Since milk sampling by trained veterinarians was not possible, and was done by the farmers, 14 farms had to be excluded because of poor sample quality and too few submitted samples. Up to 20% missing values due to poor sample quality has also been reported by Piepers et al. (2011).

While herd level information was collected by a single trained veterinarian, potential bias might have been introduced during the assessment of cow level variables by the participating farmers.

Even though our study sample size was twice as large as the sample reported in previous studies (Piepers et al., 2011), the smallest detected ORs ranged from 2.2 and 2.7 at the farm level. There may still be weaker associations that could be identified with a larger sample and these should be pursued with future research.

5. Conclusion

In this study breed and type of housing were both associated with increased *S. aureus* IMI in Swiss heifers. Rearing of heifers on specialized rearing farms, and separating pregnant heifers from younger and adults animals were protective against IMI with environmental and CNS pathogens.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2016.04.013.

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